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Biocatalytic Asymmetric Reduction of γ-Keto Esters to Access Optically Active γ-Aryl-γ-butyrolactones

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Abstract: An efficient stereoselective syntheses of a series of functionalized optically active γ-aryl-γ-butyrolactones is achieved by enzymatic asymmetric reduction of the corresponding sterically demanding γ-keto esters employing wild-type and recombinant alcohol dehydrogenases. The best stereoselectivities for the reduction via hydrogen transfer was obtained with two short chain dehydrogenases (SDRs) of complementary stereospecificity from *Aromatoleum aromaticum*, namely the Prelog-specific NADH-dependent (*S*)-1-phenylethanol dehydrogenase [(*S*)-PED] and the anti-Prelog-specific (*R*)-1-(4-hydroxyphenyl)-ethanol dehydrogenase [(*R*)-HPED], respectively. Biotransformations catalyzed by both enzymes, followed by TFA-catalyzed cyclization of the resulting γ-hydroxy esters, furnished the respective (*S*)- and (*R*)-configured products with exquisite optical purity (up to >99% ee). The synthetic value was demonstrated on preparative scale for the asymmetric bioreduction of the model compound, methyl 4-oxo-4-phenylbutanoate, affording optically pure (*S*)-γ-phenyl-γ-butyrolactone (>99% ee) in 67–74% isolated yield at 89–95% conversion depending on the applied scale.

Keywords: Biocatalysis; Biotransformations; Stereoselective Bioreductions; Alcohol Dehydrogenases; Optically Active Aromatic γ-Butyrolactones

Introduction

The dihydrofuran-2(3*H*)-one (γ-butyrolactone, GBL or γ-BL) framework is a very common structural motif in a plethora of naturally occurring products displaying a broad scope of pharmacological activities (i.e. antimicrobial, antifungal, anthelmintic, antiviral, antitumor, cytostatic, anti-inflammatory etc.), which makes them interesting lead structures for drug development.[1] In turn, optically active γ-aryl-γ-butyrolactones are considered as an important class of GBL-related compounds that serves as a valuable chiral synthons for the preparation of more complex structures of high therapeutic importance. Exemplarily, the privileged structural motif of this type is the core of many pharmacologically relevant molecules (**Figures 1**), among which the most prominent examples are: potent antitumor macrocyclic depsipeptides [(+)-cryptophycin A **I** and (+)-cryptophycin 52 **II**],[2] both isolated from strains of the terrestrial cyanobacterial genus *Nostoc* (ATCC 5371891[3] and GSV 2242,[4] respectively), and the anti-*Leishmania* agent [(–)-centrolobine[5] **III**] isolated from the heartwood of *Centrolobium robustum*[6] and from the stem of *Brosinum potabile*.[7] Moreover, several functionalized non-racemic aromatic γ-butyrolactone derivatives serve as key intermediates in the syntheses of active pharmaceutical ingredients (APIs), often providing critical sources of asymmetry. Representative compounds reaching blockbuster status in pharmaceutical industry are selective serotonin-reuptake inhibitors (SSRIs), such as sertraline[8] **IV** and fluoxetine[9] **V**, which are common antidepressants also used in alleviation of the symptoms of obsessive-compulsive disorder (OCD). Among the approved drugs originating from a γ-aryl GBL moiety are also nonsedating histamine H1-receptor antagonists (H1-RA), such as terfenadine[10] **VI** and fexofenadine[11] **VII**, which are both used for the symptomatic management of allergic rhinoconjunctivitis and chronic urticaria. In addition, the aryl GBL derived scaffold is found in the structures of two other synthetic medications, namely the antiarrhythmic agent trecetilide hemi-fumarate[12] **VIII**, which was developed for chronic treatment of reentrant cardiac arrhythmias, and the antipsychotic

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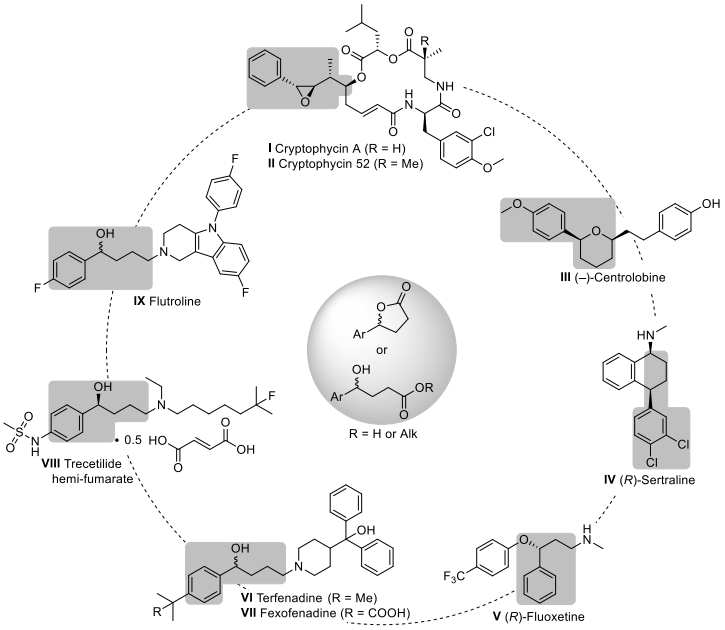
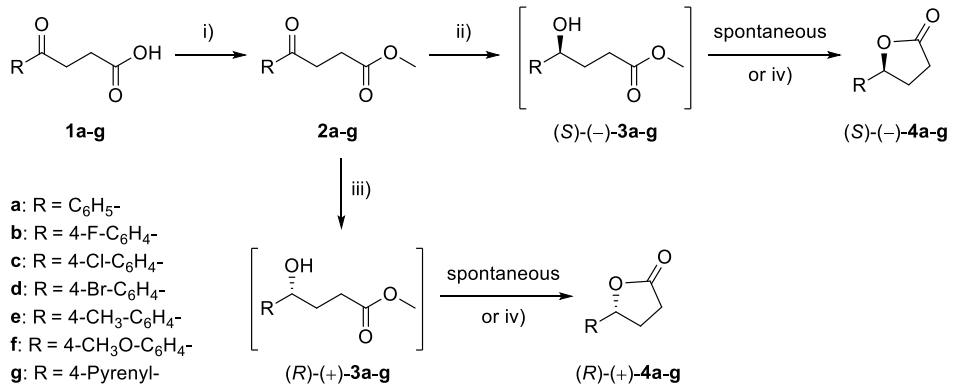


Figure 1. Examples of natural products (**I**–**III**) and pharmaceuticals (**IV**–**IX**) containing chiral γ-aryl-γ-butyrolactone core.

drug flutroline[13] **IX**, which is clinically used in the treatment of hospitalized schizophrenic patients. Due to the importance of the optically active aromatic γ-substituted γ-butyrolactones, painstaking studies have been undertaken by numerous chemo- and biocatalytic approaches aiming to prepare these compounds in enantiomerically pure form. In the last decade, the most common approaches toward the synthesis of non-racemic γ-aryl-γ-lactones was asymmetric hydrogenation and/or asymmetric transfer hydrogenation (ATH) of γ-keto esters/acids. Several elegant examples regarding the significance of both these methods have been introduced previously through various catalytic systems, mostly based on ruthenium complexes[14] as well as classical reagents such as organoboranes[15] and silanes.[16] The arsenal of other asymmetric chemical synthetic methods used encompasses: sequential hydroboration-oxidation,[17] the sequential epoxidation and Baeyer–Villiger oxidation (BVO),[18] single-step BVO of prochiral 3-substituted cyclobutanones,[19] oxidation of racemic lactols,[20] allylation of arenecarbaldehydes,[21] coupling of propargyl ethers with benzylic alcohols followed by sequential transfromation of γ-hydroxy (*Z*)-enol silanes,[22] as well as hydroacylation of aryl 1,4-keto alcohols.[23] The alternative synthetic routes towards enantiomeric γ-aryl-GBL derivatives catalyzed by chiral catalysts embrace cyclopropane ring reorganization *via* homoaldol reaction[24] or H_2O-nucleophilic cyclopropane ring-opening.[25]

In addition to organo- and metalorgano chemical methods, highly selective biocatalytic approaches have been developed in this field. Noteworthy among them are stereoselective bioreductions of γ-aryl-γ-keto acids/esters using microbial cells, including industrial baker's yeast strains[26] as well as other yeast species.[27] Furthermore, recombinant alcohol dehydrogenases (ADHs)[28] have been successfully employed. Moreover, biocatalytic methods that employ lipases or esterases as catalysts for kinetic resolutions such as lactonization of γ-aryl-γ-hydroxy esters,[29] hydrolysis of γ-phenyl-γ-butyrolactone,[30] and transesterification (acetylation) of 1-aryl-butane-1,4diols[31] or *N*-methyl-4-hydroxy-4-arylbutanamide[32] deserve attention. Less studied, but also very promising in terms of efficient preparation of 4-aryl-GBLs enantiomers, seems to be utilization of ancestral hydrolytic enzymes, such as hydroxynitrile lyase (HNL 1-NJ) and esterase (Rs EST).[33]

Although enormous progress has been made since the first chemo- or biocatalytic approaches, the synthesis of enantiomerically enriched 4-aryl-GBLs is still considered as a challenging transformation in biocatalytic synthetic chemistry. This is also due to the fact that the most common substrates used for their preparation, namely 4-oxo-4-arylbutanoates, are ketones known to be very difficult to reduce, because of the presence of two bulky substituents of the ketone moiety ("bulky-bulky" structure), which impedes access of the substrate to the active site of the enzyme. In this study, taking into account all the above challenges as well as the demand for 'greener' and more sustainable methods for the preparation of enantiomeric γ-aryl-γ-butyrolactones without using hazardous chemical reagents, we report on the development of catalytic methods based on whole-cell biocatalysts harboring overexpressed recombinant alcohol dehydrogenases (ADHs) of different origin and/or pure (*R*)- and (*S*)-specific 1-(4-hydroxyphenyl)-ethanol dehydrogenases [(*R*)-HPED and (*S*)-HPED].



Scheme 1. Chemoenzymatic synthesis of non-racemic γ-aryl-γ-butyrolactones (*S*)-(–)-**4a**–**g** and (*R*)-(+)-**4a**–**g**. Reagents and conditions: (i) **1a**–**g** (8.14 mmol), AcCl (1.2 equiv), MeOH (1 mL/0.8 mmol of substrate **1a**–**g**, ca. 10 mL) and $PhCH_3$ (in the case of **1g**), room temp., 12 h; (ii) **2a**–**g** (final concentrations 10 mM), wet cells of *E. coli*/(*S*)-PED (220 mg wet mass, 49 mg after drying, 0.638 U assayed for **2a**), 0.5 mM NADH, 0.1 M MES-KOH (pH 5.5)/2-PrOH (1000 µL, 40:60, v/v), 20–40 h, 30 ºC, 250 rpm (laboratory shaker); (iii) **2a**–**g** (final concentrations 10 mM), solution of pure (*R*)-HPED (60 µL, 0.169 U assayed for **2a**), 0.5–1.0 mM NADH, 0.1 M MES-KOH (pH 5.5)/2-PrOH (1000 µL, 90:10, v/v), 40 h, 30 ºC, 250 rpm (laboratory shaker); (iv) $TFA_{(cat.)}$, CH_2Cl_2, 1 h at 30 °C.

Results and Discussion

Herein, we report the chemoenzymatic synthesis of enantiomerically enriched (*S*)- and (*R*)-γ-aryl-γ-butyrolactones (*S*)- and (*R*)-**4a**–**g**, whereby the key step is an enzyme-catalyzed stereoselective reduction of methyl 4-oxo-4-arylbutanoates **2a**–**g** (Scheme 1). This was accomplished by employing *Escherichia coli* cells containing recombinant alcohol dehydrogenases (*E. coli*/ADHs and *E. coli*/(*S*)-PED) or by using purified enzymes from *A. aromaticum* [(*R*)-HPED and (*S*)-HPED] as biocatalysts, respectively.

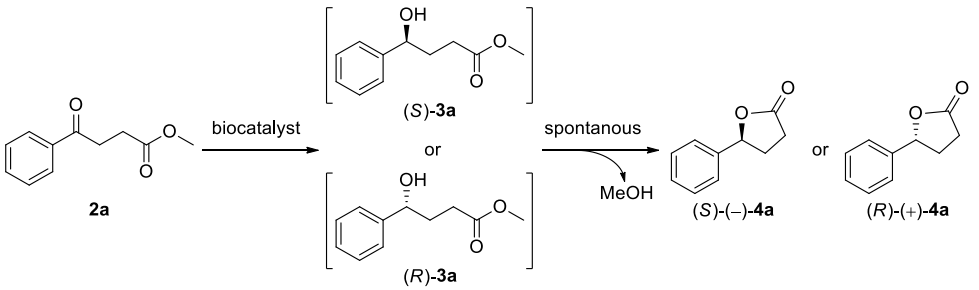
Synthesis of the Substrates

The synthesis of the prochiral ketones **2a**–**g** as well as the racemic γ-hydroxy esters *rac*-**3a**–**g** and γ-butyrolactones *rac*-**4a**–**g** was performed using standard synthetic methodologies (Scheme 1), in a 1–2-step reaction sequence, starting from cheap commercially available 4-oxo-4-arylbutanoic acids **1a**–**g**. After chemical esterification the products **2a**–**g** were isolated in 76–94% yield. Subsequently, by employing 0.50–0.75 equiv of $NaBH_4$ depending on the substrate used, racemic γ-hydroxy esters *rac*-**3a**–**g** and the corresponding γ-butyrolactones *rac*-**4a**–**g** were obtained in 10–60% and 10–46% yield ranges, respectively. Noteworthy is the fact that those compounds, which contain the bulky pyrenyl moiety **2g**, as well as those with electro-donating groups in *para*-position of the benzene ring **2e**–**f**, revealed to be less reactive under the established conditions. Therefore, they required both higher molar excess of the reducing agent as well as elongated reaction time to be fully converted into the desired products.

Biocatalytic reduction of 2a using wild-type microorganisms and recombinant alcohol dehydrogenases (ADHs)

The most prevalent biocatalyst used to stereoselectively reduce prochiral carbonyl groups in the early times of biocatalysis was baker's yeast (BY), *Saccharomyces cerevisiae*.[34] Unfortunately, as it turned out that *S. cerevisiae* cells led to sluggish bioreduction of **2a** with low to modest enantiomeric excesses (23–83% ee, see Supporting Information for details), the task was to find more potent biocatalysts possessing a wider substrate specificity and improved stereoselectivity. Consequently, reactions were run with γ-keto ester **2a** as model substrate using eight wild-type microbial strains (used as lyophilized powders) as well as six recombinant alcohol dehydrogenases (ADHs) overexpressed in *E. coli* strains [BL21(DE3) or DH5α] and employed either as lyophilized cells or wet *E. coli*/(*S*)-PED cells or in purified form [(*S*)- and (*R*)-HPED] (**Table 1**).

Table 1. Analytical-scale stereoselective reduction of **2a** (10 mM) using wild-type microorganisms (freeze-dried) or recombinant defined enzymes.



Entry	Biocatalyst	Strain	Method[a]	Conv.[b] [%]	Yield[b] [%] (**3a**/**4a**)	ee_p[c] [%] (Config. [d])
1	*Komagataella phaffi/Pichia pastoris*	ATCC 76273	A	5	0/0	N.D.[e]
2	*Pseudomonas* sp.	DSM 6978	A	24	0/9	N.D.[e]
3	*Arthrobacter* sp.	DSM 7325	A	63	6/58	86 (*S*)
4	isolate *Actinomyces* sp. SRB-AN040	FCC025	A	89	0/6	N.D.[e]
5	isolate *Actinomyces* sp. SRB-AN053	FCC027	A	4	0/0	N.D.[e]
6	isolate *Actinomyces* sp. ARG-AN024	FCC014	A	50	0/3	N.D.[e]
7	isolate ARG-AN025	FCC015	A	82	0/0	N.D.[e]
8	isolate USA-AN012	FCC021	A	69	0/3	N.D.[e]
9	*E. coli*/Lk-ADH-Lica	–	A	>99	46/54	77 (*S*)
10	*E. coli*/RasADH	–	A	92	4/85[f]	89 (*S*)
11	*E. coli*/SyADH	–	A	>99	17/83	90 (*S*)
12	*E. coli*/(*S*)-PED (gene *ped/c1A58*)	–	B	81	66/15[g]	>99 (*S*)
13	(*S*)-HPED (gene *ebA309*)	–	C	>99	80/20[g]	49 (*R*)
14	(*R*)-HPED (gene *Hped/ebA307*)	–	C	46	37/9[g]	99 (*R*)

[a] Reaction conditions. Method A: lyophilized biocatalyst (10 mg), 20 mM glucose, 0.5 mM NADH, 0.5 mM NADPH, 0.1 M Tris-HCl buffer (pH 7.5)/2-PrOH (500 µL, 90:10, v/v), 20 h, 30 ºC, 250 rpm (laboratory shaker) – anaerobic conditions. Method B: wet cells (220 mg wet cells, 49 mg after drying, 0.638 U assayed for **2a**), 0.5 mM NADH, 0.1 M MES-KOH (pH 5.5)/2-PrOH (1000 µL, 40:60, v/v), 20 h, 30 ºC, 250 rpm (laboratory shaker) – aerobic conditions. Method C: solution of pure enzyme (60 µL, 0.075 U and 0.169 U for (*S*)- and (*R*)-HPED assayed for **2a**), 0.5 mM NADH, 0.1 M MES-KOH (pH 5.5)/2-PrOH (1000 µL, 90:10, v/v), 20 h, 30 ºC, 250 rpm (laboratory shaker) – aerobic conditions.

[b] Conversion (%) (i.e., consumption of substrate **2a**) and products yields (i.e., formation of **3a**/**4a**) were determined by GC analyses using calibration curve.

[c] Determined for **4a** by HPLC analysis using a Chiralcel OD-H or (*S*,*S*)-Whelk-01 columns.

[d] Absolute configuration of optically active γ-phenyl-γ-butyrolactone (**4a**) was established by comparison of HPLC peaks elution order with our previous experiments concerning BY-mediated reactions as well as literature data.[28a and 35] Major enantiomer is shown in parentheses.

[e] Not determined.

[f] Traces (ca. 3%) of the hydrolyzed keto acid derivative **1a** were observed.

[g] To establish % ee for **4a** the lactonization of γ-hydroxy ester **3a** into γ-butyrolactone **4a** was performed by the treatment of crude mixture with catalytic amount of TFA in CH_2Cl_2 according to a procedure reported by Ramachandran *et al*.[15b]

We focused our investigations toward those microorganisms/enzymes, which have already been shown to be able to reduce "bulky-bulky" aryl alkyl ketones with high stereoselectivity. In the study we employed wild-type microorganisms,[36] recombinant ADHs, such as: *Ralstonia sp.* ADH (RasADH),[37] SyADH (originating from *Sphingobium yanoikuyae*),[38] a variant of *Lactobacillus kefir* DSM 20587[39] named Lica ADH,[40] SDRs isolated from the denitrifying bacterium *A. aromaticum* (formerly named *Azoarcus* sp. EbN1)[41] employed either as whole-cell biocatalyst, e.g. *E. coli*/(*S*)-PED (gene *ped/c1A58*)[42] or as isolated enzymes, e.g. (*S*)-HPED (gene *ebA309*) and (*R*)-HPED (gene *Hped*/*ebA307*).[43]

All the enzymatic reactions were performed with a 10 mM solution of substrate **2a** containing catalytic amounts of 0.5 mM NADH and incubated on a rotary shaker (250 rpm) at 30 °C for 20 h. For the biocatalytic hydrogen transfer experiments employing lyophilized wild-type microorganisms and *E. coli*/ADHs preparations, 0.5 mM NADPH and 20 mM glucose in a 0.1 M Tris-HCl buffer (pH 7.5) have been employed. In the case of PED/HPED-mediated biotransformations, the reaction medium was 0.1 M MES-KOH buffer (pH 5.5) and 0.5 mM NADH. In both employed strategies, the regeneration of NADH cofactors was realized by a "sacrificial

substrate-coupled" approach assay using 2-PrOH as the hydride donor at concentrations of 10 or 60% (v/v), respectively. An additional advantage of employing 2-PrOH is that it increases substrate **2a** solubility in aqueous media and is responsible for inactivation of many undesired enzymes present in the cells as well. The highest applicable concentration of 2-PrOH (60% v/v equals to 10 M) was used for *E. coli*/(*S*)-PED as this biocatalyst is highly resistant to inactivation by organic solvents.[42b] The results of the screening tests are summarized in **Table 1**.

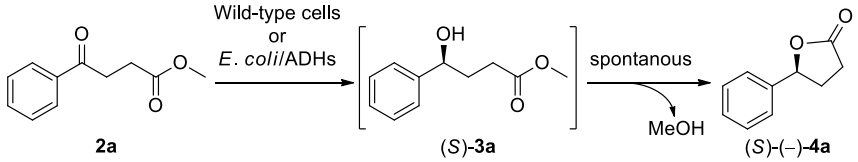
Evaluation of the selected panel of wild-type microorganisms revealed that *Arthrobacter* sp. DSM 7325 was capable of reducing **2a**, while the remaining other seven metabolized the model ketone **2a** into a complex mixture of unknown compounds with total substrate conversions reaching up to 89% value. Aforementioned *Arthrobacter* sp. catalyzed the reduction of **2a** with 63% conv. leading to optically active (*S*)-(–)-**4a** in 58% GC-yield and with 86% ee, which in fact was almost 2-fold better than obtained for BY-mediated biotransformations (see **Table S1**, entry 8 in Supporting Information). In the experiments with lyophylized *E. coli* preparations containing overexpressed ADHs (**Table 1**, entries 9–11) significantly better conversions (92–100% conv.) and optical purities (89–90% ee) were obtained with RasADH and SyADH (**Table 1**, entry 10 and 11). Furthermore, although the engineered *E. coli*/Lk-ADH-Lica also proved to be very active toward **2a**, catalyzing asymmetric bioreduction with >99% conv., it led to the formation of an almost equimolar mixture of optically active products (*S*)-**3a**/(*S*)-(–)-**4a** (46/54% GC-yield) with moderate 77% ee (**Table 1**, entry 9). Undoubtedly, the most efficient biotransformations in terms of stereoselectivity were achieved with *E. coli*/(*S*)-PED cells and pure (*R*)-HPED enzyme (**Table 1**, entry 12 and 14) with exquisite stereoselectivity in both cases (99 to >99% ee). Lower conversion was observed in the case of (*R*)-HPED (46%), whereas *E. coli*/(*S*)-PED preparation achieved 81% conv. during screening experiment (while >95% conv. was achieved in the up-scaling test – see **Table 4**). Notably, both enzymes exhibit complementary stereopreference since *E. coli*/(*S*)-PED afforded the (*S*)-configured product, while (*R*)-HPED yielded its mirror image. In contrast, pure (*S*)-HPED enzyme showed excellent results in terms of substrate conversion (>99%), but unexpectedly produced the (*R*)-configured product (*R*)-(+)-**4a**, albeit at low enantiomeric excess. Although (*S*)-HPED was confirmed to be stereoselective for (*S*)-configured alcohols in case of physiological substrates such as acetophenone, (*S*)-1-phenylethanol or *para*-hydroxyacetophenone,[43c,d] it converted **2a** preferentially to (*R*)-(+)-**4a** with 49% ee (**Table 1**, entry 13). The reversed stereopreference of (*S*)-HPED observed toward **2a** may be explained by assuming different modes of substrate binding in the active site of this enzyme than the other tested SDRs, which did not show a similar reversal and retain their excellent stereospecifity also in reducing **2a**. Exchanging the small -CH_3 group in acetophenone by the larger -$CH_2CH_2CO_2CH_3$ in **2a** apparently led to reversed substrate binding preference in (*S*)-HPED, which then presents predominantly the *si*-face of the prochiral **2a** towards hydride transfer from NADH.

All enzymes from *A. aromaticum* catalyzed the reduction of **2a** furnishing the mixtures of respective products (*S*)-**3a**/(*S*)-(–)-**4a** in favour of the γ-hydroxy ester (*S*)-**3a** formation. As the undesired uncyclized compound could interfere with the cyclized product during HPLC analysis, an additional lactonization with catalytic amount of TFA was performed. Moreover, apart from (*R*)-HPED preparation all of the tested wild-type microbial resting cells and recombinant *E. coli*/ADHs followed the Prelog rule with the hydride of NADH attacking from the *re*-face of the prochiral ketone **2a**. This has been confirmed by comparing the elution order of the respective peaks on HPLC equipped with the same chiral column as in the literature,[28a and 35] and by correlation of the optical rotation data with literature values (see Supporting Information).

It has already been stated above that reduction of **2a** and **2d** has been investigated previously[28c] employing recombinant RasADH and SyADH enzymes overexpressed in *E. coli*. Interestingly, the performed reduction afforded preferentially optically active γ-hydroxy esters instead of the respective γ-lactones. This is probably due to different reaction conditions employed (less concentrated 50 mM Tris-HCl buffer (pH 7.5) and glucose/GDH cofactor-regeneration system instead of 2-PrOH). According to the publication,[28c] the γ-hydroxy esters were obtained with (*R*)-configurations, which is in contradiction to our results. However, it has to be pointed out that the assignment of stereochemistry of the obtained optically active compounds is inconsistent since the assigned absolute configuration of (*R*)-γ-(4-bromophenyl)-γ-butyrolactone [(*R*)-**4d**] based on polarimetric analysis performed therein ($[\alpha]_D^{20} = -24.7$ (*c* 1.23, $CHCl_3$), see page: 392, line: 1) is in stark contrast with all of the previously reported data in literature. For example, for **4d** the *dextro* (+) rotation is attributed to its (*R*)-enantiomer,[23] while the *levo* (–) optical rotatory sign is characteristic for (*S*)-enantiomer.[22 and 24c] Thus, the previous data in the literature concerning the absolute configurations of enantiomeric lactones **4a** and **4d** obtained *via* reduction of the respective ketones catalyzed by RasADH and/or SyADH are incorrect.

Next, we investigated the catalytic activity of the most potent wild-type biocatalyst (*Arthrobacter* sp.) and *E. coli*/ADHs (Lk-ADH-Lica, RasADH, SyADH) in the asymmetric reduction of **2a**, with particular focus on omission of external cofactor addition and cofactor recycling (**Table 2**). Whole-cell systems often do not need addition of the external cofactors because they are already present in the cells. Furthermore, whole-cell biocatalysts in freeze-dried (lyophilized) form greatly simplify the handling. Consequently, we examined different reaction modes:

Table 2. Optimization of asymmetric reduction of **2a** (10 mM) using whole-cell biocatalyst preparations.



Entry	Biocatalyst[a]	*t* [h]	NADH [mM]	NADPH [mM]	Glucose [mM]	Conv.[b] [%]	Yield[b] [%] (**3a**/**4a**)	ee_p[c] [%]
1	*Arthrobacter* sp. DSM 7325	48	0.5	0.5	20	>99	0/90	84
2			0.5	0.5	–	>99	0/97	84
3			0.5	–	–	>99	0/93	84
4			–	–	–	36	0/30	84
5	*E. coli*/Lk-ADH-Lica	20	0.5	0.5	20	>99	46/54	77
6			0.5	0.5	–	96	6/87	77
7			0.5	–	–	>99	9/91	76
8			–	–	–	97	9/85	80
9	*E. coli*/RasADH	20	0.5	0.5	20	92	4/85	89
10			0.5	0.5	–	96	5/91	85
11			0.5	–	–	96	3/93	89
12			–	–	–	96	5/81	92
13	*E. coli*/SyADH	20	0.5	0.5	20	>99	17/83	90
14			0.5	0.5	–	95	5/84	86
15			0.5	–	–	95	4/80	91
16			–	–	–	88	11/63	92

[a] Reaction conditions: lyophilized biocatalyst (10 mg), 0.5 mM NADH (or without), 0.5 mM NADPH (or without), 20 mM glucose (or without), 0.1 M Tris-HCl buffer (pH 7.5)/2-PrOH (500 µL, 90:10, v/v), 20–48 h, 30 ºC, 250 rpm (laboratory shaker) – anaerobic conditions.

[b] Conversion (%) (i.e., consumption of substrate **2a**) and products yields (i.e., formation of **3a**/**4a**) were determined by GC analyses using calibration curve.

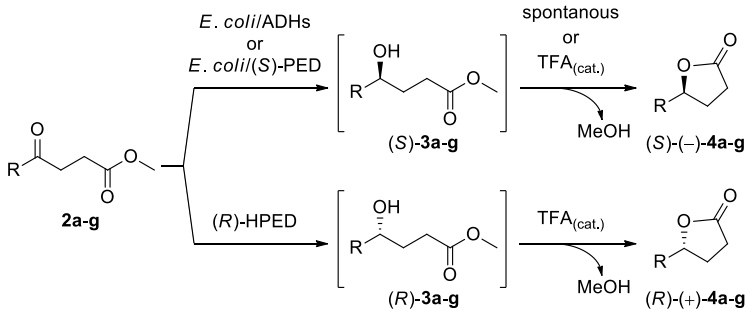
[c] Determined for **4a** by HPLC analysis using a Chiralcel OD-H column or (*S*,*S*)-Whelk-01 columns.

(i) with the addition of NADH, NADPH and glucose, (ii) without glucose, (iii) without glucose and NADPH, and finally (iv) without all aforementioned compounds. In addition, for the bioreduction of **2a** catalyzed by *Arthrobacter* sp. DSM 7325 the time of the reaction was elongated 2-fold to reach >99% conv. Inspection of **Table 2** clearly shows that in almost all cases external addition of cofactors and glucose was not improving the process, except in case of *Arthrobacter* sp. DSM 7325, for which addition of 0.5 mM NADH increased conversion from 36% to >99%. However, due to spontaneous lactonization of the reduced **3a** to **4a**, *Arthrobacter* sp. DSM 7325 turned out to be the only full step-economical biocatalyst yielding enantiomeric lactone (*S*)-(–)-**4a** in a one-pot operation strategy in 90–97% yield and 84% ee, respectively. Furthermore, in terms of the stereoselectivity of bioreduction, the differences were also negligible (2–7% ee), albeit in favor of the reactions carried out without additives, what only proved that this transformations can be carried out under NAD(P)H-free conditions. Undoubtedly, the lyophylized *E. coli* cells containing RasADH and SyADH turned out to be the most potent biocatalysts with regard to optical purity of the obtained lactone (*S*)-(–)-**4a** (92% ee) (**Table 2**, entry 12 and 16), and thus were selected along with *E. coli*/(*S*)-PED and pure (*R*)-HPED for further investigations.

Biocatalytic reduction of 2a–f using recombinant alcohol dehydrogenases (ADHs)

In the next step, to demonstrate the utility of the library of the selected enzymes as biocatalysts in asymmetric reduction of γ-aryl-γ-keto esters, we have extended the substrate scope to other "bulky-bulky" compounds **2b–f** possessing different substitution patterns in *para*-position of the benzene ring (**Table 3**). Moreover, to verify if these biocatalysts are able to perform transformations of sterically highly demanding ketones, reduction of the substrate with pyrenyl moiety **2g** was also investigated. Then biotransformations were conducted with: *E. coli*/RasADH, *E. coli*/SyADH, *E. coli*/(*S*)-PED and (*R*)-HPED, using three different synthetic procedures as applied before (Methods A–C) or the protocol with isolated (*R*)-HPED enzyme, but after minor modification (Method D). The assignment of the stereochemistry to all the obtained enantiomeric products of ADHs- and PED/HPED-catalyzed bioreductions was accomplished by comparing elution profiles on HPLC with a chiral phase using the reference materials obtained in the preparative-scale BY-mediated biotransformations of **2a** and **2c–d** (see Supporting Information).

Table 3. Analytical-scale studies on (ADHs)-catalyzed reductions of "bulky-bulky" γ-aryl-γ-keto esters **2a**–**g** (10 mM).



Entry	Substrate	R	ADHs	Method[a]	*t* [h]	Conv.[b] [%]	Yield[b] [%] (**3a**/**4a**)	ee$_p$[c] [%] (Config.[d])
1	**2a**	C_6H_5	*E. coli*/RasADH	A	20	96	5/81	92 (*S*)
2			*E. coli*/SyADH	A	20	88	11/63	92 (*S*)
3			*E. coli*/(*S*)-PED	B	40	90	68/8	>99 (*S*)
4			(*R*)-HPED	C	40	57	36/21	99 (*R*)
5				D	40	67	47/20	99 (*R*)
6	**2b**	4-F-C_6H_4	*E. coli*/RasADH	A	20	98	8/90	82 (*S*)
7			*E. coli*/SyADH	A	20	96	8/88	7 (*S*)
8			*E. coli*/(*S*)-PED	B	20	>99	86/14	99 (*S*)
9			(*R*)-HPED	C	40	84	71/13	98 (*R*)
10	**2c**	4-Cl-C_6H_4	*E. coli*/RasADH	A	20	96	9/87	55 (*S*)
11			*E. coli*/SyADH	A	20	90	0/81	53 (*R*)
12			*E. coli*/(*S*)-PED	B	20	98	72/26	>99 (*S*)
13			(*R*)-HPED	C	40	80	60/20	93 (*R*)
14	**2d**	4-Br-C_6H_4	*E. coli*/RasADH	A	20	98	0/91	62 (*S*)
15			*E. coli*/SyADH	A	20	95	0/85	58 (*R*)
16			*E. coli*/(*S*)-PED	B	20	>99	68/32	>99 (*S*)
17			(*R*)-HPED	C	40	36	25/11	74 (*R*)
18				D	40	44	28/16	76 (*R*)
19	**2e**	4-CH_3-C_6H_4	*E. coli*/RasADH	A	20	>99	0/98	76 (*S*)
20			*E. coli*/SyADH	A	20	>99	0/98	50 (*S*)
21			*E. coli*/(*S*)-PED	B	20	96	78/18	46 (*S*)
22			(*R*)-HPED	C	40	35	24/11	76 (*R*)
23				D	40	40	30/10	77 (*R*)
24	**2f**	4-CH_3O-C_6H_4	*E. coli*/RasADH	A	20	87	0/87	74 (*S*)
25			*E. coli*/SyADH	A	20	55	0/50	52 (*S*)
26			*E. coli*/(*S*)-PED	B	40	74	50/24	75 (*S*)
27			(*R*)-HPED	C/D	40	0	0/0	N.D.[e]
28	**2g**	4-Pyrenyl[f]	*E. coli*/RasADH	A	20	0	0/0	N.D.[e]
29			*E. coli*/SyADH	A	20	0	0/0	N.D.[e]
30			*E. coli*/(*S*)-PED	B	20	0	0/0	N.D.[e]
31			(*R*)-HPED	C	20	0	0/0	N.D.[e]

[a] Reaction conditions. Method A: **2a**–**g** (final concentrations 10 mM), lyophilized biocatalyst (10 mg), 0.1 M Tris-HCl buffer (pH 7.5)/2-PrOH (500 µL, 90:10, v/v), 20 h, 30 ºC, 250 rpm (anaerobic). Method B: **2a**–**g** (final concentrations 10 mM), wet cells (220 mg wet cells, 49 mg after drying, 0.638 U assayed for **2a**), 0.5 mM NADH, 0.1 M MES-KOH (pH 5.5)/2-PrOH (1000 µL, 40:60, v/v), 20–40 h, 30 ºC, 250 rpm (aerobic). Method C: **2a**–**g** (final concentrations 10 mM), solution of pure enzyme (60 µL, 0.169 U (*R*)-HPED assayed for **2a**), 0.5 mM NADH, 0.1 M MES-KOH (pH 5.5)/2-PrOH (1000 µL, 90:10, v/v), 20–40 h, 30 ºC, 250 rpm (aerobic). Method D: **2a, d**–**g** (final concentrations 10 mM), solution of pure enzyme (60 µL, 0.169 U (*R*)-HPED assayed for **2a**), 1.0 mM NADH, 0.1 M MES-KOH (pH 5.5)/2-PrOH (1000 µL, 90:10, v/v), 40 h, 30 ºC, 250 rpm (aerobic).

[b] Conversion values (%) (i.e., consumption of substrate **2a**–**f**) and products yields (i.e., formation of **3a**–**f**/**4a**–**f**) were determined by GC analyses using calibration curves.

[c] Determined for optically active γ-aryl-γ-butyrolactones **4a**–**g** by chiral HPLC analysis using (*S*,*S*)-Whelk-01 or Chiralcel OD-H columns, respectively.

[d] Absolute configuration of **4a**–**f** established by comparison of HPLC peaks elution order and optical rotation signs with literature data.[28a and 35] Major enantiomer is shown in parentheses.

[e] Not determined.

[f] Additional portion of DMSO (30 µL) was added in order to increase substrate **2g** solubility.

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Detailed analysis of the data revealed that the most potent enzyme preparation in terms of the conversion as well as stereoselectivity was *E. coli*/(*S*)-PED. The reductions of γ-aryl-γ-keto esters **2a**–**f** catalyzed by these cells provided (*S*)-configured γ-aryl-γ-hydroxy esters (*S*)-**3a**–**f**, which after treatment with TFA produced the corresponding lactones (*S*)-(–)-**4a**–**f** with moderate (46–52% ee) to mostly excellent optical purity (99 to >99% ee). For biotransformations of model substrate **2a** as well as derivatives **2b**–**d** possessing electron-withdrawing groups (–F, –Cl, and –Br) situated in the *para*-position of the phenyl substituent almost no traces of the (*R*)-enantiomers were detected by HPLC, which demonstrates the excellent selectivity exerted by *E. coli*/(*S*)-PED. Out of six ketones **2a**–**f**, three of them, namely **2a** and **2c**–**d**, were transformed into optically pure lactones (*S*)-(–)-**4a** and (*S*)-(–)-**4c**–**d**, while in the case of *para*-fluoro-substituted derivative **2b** the enantiomeric purity of (*S*)-(–)-**4b** was found to be 99%. For substrates substituted with electron withdrawing groups (**2b**–**d**) *E. coli*/(*S*)-PED gave the target products with >98% conv. after 20 h. For the reference substrate **2a**, we observed 81% conv. after 20 h and 90% conv. after 40 h and 98% in the up-scaling experiment. On the other hand, lower conversion and especially diminished stereoselectivity were observed for compounds **2e**–**f** bearing electron-donating groups (–CH_3 and –OCH_3) (**Table 3**, entries 21 and 26). This result is consistent with previous studies on *E. coli*/(*S*)-PED, where bioreduction of *para*-OH and *para*-NH_2 substituted acetophenones resulted with decreased stereoselectivity (respective ee of 90 and 0%)[43a] as well as relative specific activities in bioreduction of acetophenone and 4-hydroxyacetophenone for HPEDs.[43d]

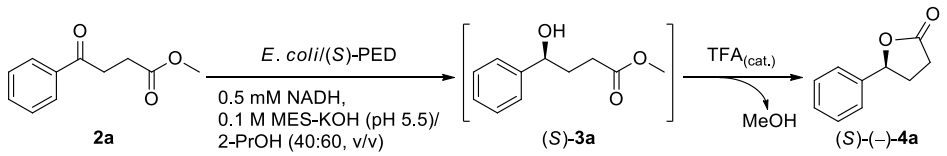
Subsequently, the use of (*R*)-HPED provided the opposite (*R*)-enantiomers of the respective mixtures of γ-aryl-(-keto esters/-lactones) (*R*)-**3a**–**f**/(*R*)-(+)-**4a**–**f** with mostly excellent stereoselectivity allowing to obtain exquisite enantiomeric excesses of up to 99% for (*R*)-(+)-**4a** and 98% for (*R*)-(+)-**4b** after additional TFA-catalyzed lactonisation procedure. While substrates **2a**–**b** were transformed with high stereoselectivity, the other substrates led to the formation of the target lactones (*R*)-(+)-**4c**–**e** with enantiomeric purities between 74 and 93% ee. From the panel of screened substrates, **2b** and **2c** were reduced with >80% conv. after 40 h while in other cases the conversion was lower. Interestingly, (*R*)-HPED failed to catalyze the reduction of **2f**. As the six ketones **2a**–**f** are of comparable size, the loss of reactivity was probably due to unfavorable electronic effects of the methoxy group present in **2f**.

In contrast to PED/HPED-mediated biotransformations of **2a**–**f**, the reductions catalyzed by *E. coli* cells harboring the respective RasADH and SyADH recombinant enzymes were less efficient. Despite very high 87–100% conv. accomplished in a reasonable time scale (20 h), the formation of enantiomerically enriched lactones **4a**–**f** proceeded in a moderate stereoselective manner (50–92% ee). We were puzzled by the result of diminished optical purity value for (*S*)-(–)-**4b** (7% ee) obtained from the reaction catalyzed by SyADH especially as fluorine is a bioisostere of the hydrogen atom. The unexpected low stereoselectivity of this reaction for **2b** was ascertained by three independent experiments under the same conditions. Explanation of this finding is rather difficult, although it is possible that greater lipophilicity of fluorine as well as its strong electronic features may play a detrimental role in specific interactions in a catalytic cavity of the SyADH impeding efficient stereorecognition. Nevertheless, the observed huge difference in stereochemical outcome of transformation of this particular ketone **2b** can be ascribed by distinguished substrate specificity of the tested SyADH as well. Notably, a remarkable drop in stereoselectivity was found in the bioreduction of *para*-chloro- **2c** and *para*-bromo-substituted **2d** derivatives using both *E. coli*/ADHs, together with a different selectivity pattern as it turned out that these biocatalysts displayed opposite stereopreference on compounds **2c**–**d** leading to the formation of (*S*)-(–)-**4c** (55% ee) and (*S*)-(–)-**4d** (62% ee) in the case of RasADH, and (*R*)-(+)-**4c** (53% ee) and (*R*)-(+)-**4d** (58% ee) in the case of SyADH.

Interestingly, the results with **2a**–**f** revealed that the reactions mediated by *E. coli*/RasADH and *E. coli*/SyADH provided predominantly enantiomeric γ-lactones **4a**–**g** over the respective γ-hydroxy esters **3a**–**g**. Currently, we are lacking a plausible explanation for this observation as the lactone formation rate was reported to be largely pH dependent,[44] and its promotion may be expected when acidic conditions are applied. In this case, when we employed MES-KOH buffer of slightly acidic pH 5.5 as the medium for PED/HPED-based reactions, it turned out that hydroxyl products are formed in favor, whereas in Tris-HCl buffer pH 7.5 designed for *E. coli*/ADHs-based reactions, the desired lactones were observed almost as sole products. This may suggest that it is not the pH of the buffer, which plays a decisive role, but maybe the local pH in the cells or interaction with another enzyme that promotes lactonization. Investigation of the substrate range also revealed that all of the tested enzyme preparations are completely inactive towards the sterically demanding ketone **2g** (**Table 3**, entries 28–31). The bulkiness of the pyrenyl substituent likely prevents fitting the substrate into the enzymes active sites, and thus suitable variants may have to be identified to overcome this circumstances in the future.

Having an optimal biocatalytic system in hand, we performed further scale-up experiments with **2a** (0.3 mmol, 0.6 mmol and 4.2 mmol) in order to prove that *E. coli*/(*S*)-PED cells displayed not only high selectivity toward prochiral γ-aryl-γ-keto esters, but also sufficient productivity (**Table 4**). The preparative-scale trials showed that under the best reaction conditions identified, the *E. coli*/(*S*)-PED biocatalyst allowed high level of consumption of

Table 4. Preparative-scale *E. coli*/(*S*)-PED-catalyzed bioreduction of methyl 4-oxo-4-phenylbutanoate (**2a**).



Entry	Scale [mmol][a]	Conv.[b] [%]	GC-yield[c] [%] (**3a**/**4a**)	Isolated yield[c] [%]	ee_p[d] [%]
1	0.3	96	66/30	62	98
2	0.6	95	70/25	74	>99
3	2.1	>98[e]	N.D.[f]	N.D.[f]	N.D.[f]
4	4.2	89	75/14	67	>99

[a] Reaction conditions for 0.3–0.6 mmol scale. **2a** (0.3 mmol or 0.6 mmol), *E. coli*/(*S*)-PED wet cells (6.6 g, activity 19.14 U relative to **2a** or 13.2 g, activity 38.28 U relative to **2a**)), 0.5 mM NADH, 0.1 M MES-KOH (pH 5.5)/2-PrOH (30 mL or 60 mL, 40:60, v/v), 40 h, 30 ºC, 250 rpm (laboratory shaker) – aerobic conditions; $TFA_{(cat.)}$, CH_2Cl_2 (15 mL or 30 mL), 1 h at 30 °C. Reaction conditions for 2.1-4.2 mmol scale (fed-batch mode). initial loading of **2a** (2.1 mmol), **2a** addition after 20 h (2.1 mmol), *E. coli*/(*S*)-PED wet cells (46.2 g, activity 133.98 U relative to **2a**), 0.5 mM NADH, 0.1 M MES-KOH (pH 5.5)/2-PrOH (210 mL, 40:60, v/v), 80 h (total time), 30 ºC, 250 rpm (laboratory shaker) – aerobic conditions; $TFA_{(cat.)}$, CH_2Cl_2 (50 mL), 5 h at 30 °C.

[b] Conversion (%) (i.e., consumption of substrate **2a**) and products yields (i.e., formation of **3a**/**4a**) were determined by GC analyses using calibration curve.

[c] Isolated yield after column chromatography on SiO_2.

[d] Determined for optically active (*S*)-(–)-**4a** by chiral HPLC analysis using (*S*,*S*)-Whelk-01 column.

[e] Conversion (%) determined by HPLC analysis from the samples directly withdrawn from crude reaction mixture.

[f] Not determined.

the starting material **2a** (89–96% conv.) with GC-yields reaching a range of 66–75% for (*S*)-**3a** and 14–30% for (*S*)-(–)-**4a**, respectively. After TFA-catalyzed lactonization of the crude mixtures, the product isolation led to 30 mg (62% isolated yield), 72 mg (74% isolated yield) and 457 mg (67% isolated yield) of (*S*)-(–)-**4a** depending on the applied scale. It is worth noting that the bioreduction of **2a** conducted on a 4.2 mmol scale (807 mg of the substrate **2a**) was performed in a fed-batch mode for 80 h of total reaction time. This attempt allowed us to clarify reliability of the up-scaling procedure as well as led to a significant drop in overall employed biocatalyst to substrate ratio. The most important fact is that **2a** was reduced with perfect enantioselectivity, thus (*S*)-(–)-**4a** being obtained in highly enantiopure form (form 98% ee to >99% ee), accurately preserving stereoselectivity of the *E. coli*/(*S*)-PED from the analytical-scale reactions.

Conclusion

In order to expand the bioorganic synthesis toolbox for the preparation of enantiomerically pure γ-aryl-γ-butyrolactones, various whole-cell biocatalysts, including: (i) baker's yeast, (ii) wild-type microorganisms with carbonyl reductase activity as well as (iii) *E. coli* cells harboring recombinant alcohol dehydrogenases designed for "bulky-bulky" substrates have been tested in the asymmetric reduction of the respective γ-aryl-γ-keto esters.

Additionally, (iv) purified oxidoreductive enzymes were employed. From the above-listed biocatalysts, the most outstanding selectivity in hydrogen transfer to the carbonyl group was obtained with *E. coli* cells containing recombinant NADH-dependent (*S*)-selective 1-phenylethanol dehydrogenase from the denitrifying bacterium *A. aromaticum* strain EbN1, namely *E. coli*/(*S*)-PED. Depending on the type of substitution pattern in the tested prochiral substrates, significant differences in the stereochemical outcome were observed. Using a one-pot two-step direct bioreduction-lactonization reaction sequence catalyzed by a system composed of *E. coli*/(*S*)-PED biocatalysis and TFA-dependent chemical conversion, enantiomeric (*S*)-(–)-γ-aryl-γ-butyrolactones have been obtained as targets with 67–100% conv., and mostly with excellent stereoselectivities (i.e. reaching 99 to >99% ee for phenyl-like as well as *para*-fluoro, *para*-chloro and *para*-bromo derivatives). Moderate optical purities (46–75% ee) were obtained with *para*-methyl and *para*-methoxy compounds. The preparative-scale studies with 4-oxo-4-phenylbutanoate (0.6–4.2 mmol) accomplished the optically pure (*S*)-γ-phenyl-γ-butyrolactone (>99% ee) in high 67–74% isolated yields. The second enzyme preparation investigated that was identified to be highly useful for stereoselective reduction of the γ-keto esters is (*R*)-HPED, which allowed to obtain (*R*)-configured γ-phenyl-γ-butyrolactone and its derivative with a fluorine atom at the *para*-position with nearly excellent enantioenrichment (98–99% ee). For the other substrates studied (*R*)-HPED led to

enantiomeric excesses between 76% and 93% (i.e. 93% ee for *para*-Cl, 76% ee for *para*-Br, and 77% ee for *para*-Me), while for the *para*-OMe derivative the reaction was fully suppressed. Interestingly, in the case of bioreductions catalyzed by *E. coli*/RasADH and *E. coli*/SyADH a mutual reversed stereochemistry was found only when methyl 4-(4-chloro-phenyl)- and 4-(4-bromo-phenyl)-4-oxobutanoates were employed as substrates. In these cases, *E. coli*/RasADH preserved Prelog stereospecificity leading to (*S*)-configured lactones, whereas *E. coli*/SyADH catalyzed the formation of the respective (*R*)-configured counterparts.

Experimental Section

General experimental methods: Reagents and solvents were purchased from various commercial sources (Sigma Aldrich, Alfa Aesar, POCH) and were used without further purification. High-performance liquid chromatography HPLC-grade solvents (*n*-hexane, 2-PrOH and EtOH) were purchased from POCH (Poland). Whole cell biocatalysts (WCBs) including cells of wild type strains[36] and *E. coli* cells containing overexpressed recombinant alcohol dehydrogenases (ADHs) *Ralstonia sp.* ADH (RasADH, pEG 105),[37c] SyADH (originating from *Sphingobium yanoikuyae*, pEG 53)[38] have been prepared as previously reported. All 1-(4-hydroxyphenyl)-ethanol dehydrogenases from *Aromatoleum aromaticum* were from the Jerzy Haber Institute of Catalysis and Surface Chemistry, PAS and have been prepared following the methodology previously described [see: *E. coli*/(*S*)-PED[42a] (also known as PED), (*S*)-HPED[43c] (also known as EbA309) and (*R*)-HPED[43d] (also known as Hped or ChnA)]. Evaporation of the solvent residues was performed at reduced pressure by means of Büchi rotary evaporator and high-vacuum oil pump at *p*=0.05 mmHg. Melting points, uncorrected, were determined with a commercial apparatus (Thomas-Hoover "UNI-MELT" capillary melting point apparatus) on samples contained in rotating glass capillary tubes open on one side (1.35 mm inner diam. and 80 mm length). Analytical thin-layer chromatography was carried on TLC aluminum plates (Merck) covered with silica gel of 0.2 mm thickness film containing a fluorescence indicator green 254 nm (F_{254}), and using UV light as a visualizing agent. Preparative separations were carried out by column chromatography using thick-walled glass columns and silica gel (230–400 mesh) with grain size 40–63 μm purchased from Merck, Germany. The chromatographic analyses (GC) were performed with a Agilent Technologies 6890N instrument equipped with a flame ionization detector (FID) and fitted with HP-50+ (30 m) semipolar column (50% phenyl–50% methylpolysiloxane); Helium (2 mL/min) was used as carrier gas; retention times (t_R) are given in minutes under these conditions. The enantiomeric excesses (% ee) of kinetic resolution products were determined by HPLC analyses performed on Shimadzu CTO-10ASV chromatograph equipped with STD-20A UV detector and chiral columns as follows: Chiralcel OD-H (4.6 mm × 250 mm, coated on 5 μm silica gel grain size, from Daicel Chemical Ind., Ltd.) or Chiralpak AD-H (4.6 mm × 250 mm, coated on 5 μm silica gel grain size, from Daicel Chemical Ind., Ltd.) or (*S,S*)-Whelk-O 1 (4.6 mm × 250 mm, coated on 5 μm silica gel grain size, from Regis Pirkle Technologies, INC.) all of them equipped with a pre-column (4 mm × 10 mm, 5 μm) using mixtures of *n*-hexane/2-PrOH or *n*-hexane/EtOH as mobile phase in the appropriate ratios given in experimental section [both the mobile phase composition as well as the flow rate were fine tuned for each analysis (see **Table S3**)]; the wavelength of UV detection was set at 220 nm for γ-hydroxybutyrates *rac*-**3a**–**g** and 210 nm for γ-butyrolactones *rac*-**4a**–**g**; the HPLC analyses were executed in an isocratic and isothermal (30 °C) manner. Optical rotations ([α]) were measured with a PolAAr 32 polarimeter in a 2 dm long cuvette using the sodium D line (λ=589 nm); the units of the specific rotation are: (deg×mL)/(g×dm). ^{1}H NMR (500 MHz), ^{13}C NMR (126 MHz) and ^{19}F NMR (470 MHz) spectra were recorded on a Varian NMR System 500 MHz spectrometer; ^{1}H, ^{13}C and ^{19}F chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent signals [$CDCl_3$, δ_H (residual $CHCl_3$) 7.26 ppm, δ_C 77.16 ppm] or internal $CFCl_3$ reference set at 0 ppm. Chemical shifts are quoted as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br s (broad singlet); coupling constants (J) are reported in Hertz (Hz). Mass spectrometry was recorded on Micro-mass ESI Q-TOF spectrometer with MSI concept 1H (EI, 70eV ionization) for MS analysis and on Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer, ESI source: electrospray with spray voltage 4.00 kV for FTMS analysis; all samples were prepared by dilution with MeOH (0.5 mL) and additives of mixtures of $CH_3CN/MeOH/H_2O$ (50:25:25, v/v/v) + 0.5% formic acid each.

General procedure for the synthesis of methyl 4-oxo-4-arylbutanoates 2a–g

To a solution of the appropriate 4-oxo-4-arylbutanoic acid **1a**–**g** (8.14 mmol) in MeOH (1 mL/0.8 mmol of substrate, ca. 10 mL) acetyl chloride (1.2 equiv) was added dropwise and the reaction mixture was stirred at room temperature for 12 h. [Attention: in the case of **1g** additional portion of $PhCH_3$ (20 mL) was added to increase solubility of the substrate]. After completion of the reaction [according to TLC indications developed in hexane/AcOEt (1:1 v/v) as a mobile phase], the crude mixture was concentrated under vacuum to remove MeOH, the obtained slurry was dissolved in CH_2Cl_2 (50 mL), washed with portions of saturated $NaHCO_3$ (2 × 30 mL) and brine (2 × 30 mL), respectively. Next, an aqueous phase was back-extracted with CH_2Cl_2 (1 × 50 mL), and the combined organic layer was dried over anhydrous $MgSO_4$. After filtration of the drying agent, the permeate was concentrated under reduced pressure to obtain the corresponding methyl 4-oxo-4-arylbutanoate **2a**–**g** in an excellent purity allowing them to be used in the next step without further purification. In the case of crude **2g** additional purification procedure employing recrystalization from the mixture of hexane/AcOEt (1:1 v/v) was necessary to obtain a pure product.

Methyl 4-oxo-4-phenylbutanoate (2a). Yield 84% (1.31 g); light yellow liquid; R_f [hexane/AcOEt (1:1 v/v)] 0.84; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.75 (t, J=6.6 Hz, 2H), 3.31 (t, J=6.6 Hz, 2H), 3.69 (s, 3H), 7.42–7.48 (m, 2H), 7.52–7.58 (m, 1H), 7.94–8.00 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 28.0, 33.4, 51.8, 128.0, 128.6, 133.2, 136.5, 173.3, 198.0; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{13}O_3^+$ m/z: 193.0860, Found 193.0706; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{13}O_3^+$ m/z: 193.08592, Found 193.08623; GC [200–260 (10 °C/min)]: t_R=2.120 min.

Methyl 4-(4-fluorophenyl)-4-oxobutanoate (2b). Yield 92% (1.57 g); white solid; mp 52–54 °C (CH_2Cl_2) [lit.[45] 51–52 °C (CH_2Cl_2)]; R_f [hexane/AcOEt (1:1 v/v)] 0.82; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.76 (t, J=6.6 Hz, 2H), 3.28 (t, J=6.6 Hz, 2H), 3.70 (s, 3H), 7.09–7.16 (m, 2H), 7.97–8.03 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 28.0, 33.3, 51.8, 115.8 (d, J_{C-F}=21.5 Hz), 130.7 (d, J_{C-F}=8.8 Hz), 133.0 (d, J_{C-F}=2.9 Hz), 165.8 (d, J_{C-F}=254.1 Hz), 173.3, 196.4; ^{19}F NMR (470 MHz, $CDCl_3$): δ –105.05 (tt, J=8.39, 5.3 Hz); MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{12}FO_3^+$ m/z: 211.0765, Found 211.0766; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{12}FO_3^+$ m/z: 211.07650, Found 211.07671; GC [200–260 (10 °C/min)]: t_R=1.928 min.

Methyl 4-(4-chlorophenyl)-4-oxobutanoate (2c). Yield 94% (1.73 g); white solid; mp 52–55 °C (CH_2Cl_2) [lit.[46]

51.6–52.8 °C (Et_2O)]; R_f [hexane/AcOEt (1:1 v/v)] 0.64; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.76 (t, J=6.6 Hz, 2H), 3.27 (t, J=6.6 Hz, 2H), 3.70 (s, 3H), 7.39–7.47 (m, 2H), 7.88–7.95 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 27.9, 33.3, 51.9, 128.9, 129.4, 134.8, 139.7, 173.2, 196.8; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{12}ClO_3^+$ m/z: 227.0470, Found 227.0470; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{12}ClO_3^+$ m/z: 227.04695, Found 227.04716; GC [200–260 (10 °C/min)]: t_R=2.969 min.

Methyl 4-(4-bromophenyl)-4-oxobutanoate (2d). Yield 86% (1.89 g); white solid; mp 49–53 °C (CH_2Cl_2) [lit.[47] 51–52 °C (H_2O)]; R_f [hexane/AcOEt (1:1 v/v)] 0.78; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.76 (t, J=6.6 Hz, 2H), 3.27 (t, J=6.6 Hz, 2H), 3.70 (s, 3H), 7.58–7.63 (m, 2H), 7.81–7.86 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 27.9, 33.3, 51.9, 128.4, 129.5, 131.9, 135.2, 173.2, 197.0; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{12}BrO_3^+$ m/z: 270.9965, Found 270.9892; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{12}BrO_3^+$ m/z: 270.99643, Found 270.99646; GC [200–260 (10 °C/min)]: t_R=3.718 min.

Methyl 4-(4-methylphenyl)-4-oxobutanoate (2e). Yield 81% (1.37 g); white solid; mp 46–47 °C (CH_2Cl_2) [lit.[48] 51–53 °C (AcOEt/petroleum ether)]; R_f [hexane/AcOEt (1:1 v/v)] 0.70; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.40 (s, 3H), 2.75 (t, J=6.9 Hz, 2H), 3.29 (t, J=6.9 Hz, 2H), 3.69 (s, 3H), 7.22–7.28 (m, 2H), 7.85–7.90 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.6, 28.1, 33.3, 51.8, 128.1, 129.3, 134.1, 144.0, 173.4, 197.6; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{15}O_3^+$ m/z: 207.1016, Found 207.0889; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{15}O_3^+$ m/z: 207.10157, Found 207.10163; GC [200–260 (10 °C/min)]: t_R=2.640 min.

Methyl 4-(4-methoxyphenyl)-4-oxobutanoate (2f). Yield 86% (1.56 g); white solid; mp 51–53 °C (CH_2Cl_2) [lit.[49] 48.1–49.4 °C (MeOH/AcOEt)]; R_f [hexane/AcOEt (1:1 v/v)] 0.76; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.74 (t, J=6.9 Hz, 2H), 3.26 (t, J=6.9 Hz, 2H), 3.69 (s, 3H), 3.86 (s, 3H), 6.90–6.95 (m, 2H), 7.93–7.98 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 28.1, 33.0, 51.8, 55.5, 113.7, 129.6, 130.3, 163.6, 173.5, 196.5; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{15}O_4^+$ m/z: 223.0965, Found 223.0919; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{15}O_4^+$ m/z: 223.09649, Found 223.09660; GC [200–260 (10 °C/min)]: t_R=3.907 min.

Methyl 4-oxo-4-(pyren-1-yl)butanoate (2g). Yield 76% (1.98 g); yellow solid; mp 111–111.5 °C (hexane/AcOEt); R_f [hexane/AcOEt (1:1 v/v)] 0.80; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.94 (t, J=6.5 Hz, 2H), 2.65 (t, J=6.5 Hz, 2H), 2.77 (s, 3H), 7.22–7.27 (m, 1H), 7.31–7.36 (m, 1H), 7.39–7.44 (m, 2H), 7.45–7.52 (m, 3H), 7.65–7.70 (m, 1H), 7.85–7.91 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 28.2, 36.8, 51.5, 123.5, 124.0, 124.4, 124.4, 126.0, 126.5, 126.6, 126.7, 127.1, 128.2, 129.3, 129.4, 129.9, 130.6, 132.0, 133.1, 173.0, 202.8; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{21}H_{17}O_3^+$ m/z: 317.1173, Found 317.0753; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{21}H_{17}O_3^+$ m/z: 317.11722, Found 317.11728; GC [260 (const.)]: t_R=Not Found.

General procedure for the synthesis of racemic γ-hydroxy esters *rac*-3a–g and γ-butyrolactones *rac*-4a–g

To a solution of the appropriate γ-keto ester **2a**–**g** (5.20 mmol) in a mixture of Et_2O/MeOH (18 mL, 1:1 v/v) was added a solution of $NaBH_4$ (0.50 equiv for **2a**–**d** and 0.75 equiv for **2e**–**g**) in H_2O (400 µL) with ice cooling. [Attention: in the case of **2g** additional portion of $PhCH_3$ (10 mL) was added to increase solubility of the substrate]. After stirring at 0–5 °C for 2 h (for **2a**–**d**) and 6 h (for **2e**–**f**), additional portion of Et_2O (20 mL) was added and the excess of $NaBH_4$ was decomposed with cold 1 % aqueous solution of $NaHCO_3$ (30 mL). The reaction mixture was extracted with Et_2O (3 × 30 mL), washed with H_2O (100 mL), and dried over anhydrous $MgSO_4$. After removal of the drying agent, the solvent was evaporated to give a yellowish oil, which was chromatographed on silica gel eluting with the appropriate mixture of CH_2Cl_2/AcOEt (90:10, v/v) as an eluent to afford the respective γ-hydroxy ester *rac*-**3a**–**f** and γ-butyrolactone *rac*-**4a**–**f**. [Attention: in the case of **2g** the purification procedure was slightly modified. After the completion of the reaction (6 h) the precipitated dark-yellowish solid was filtered off under suction and washed with portion of $PhCH_3$ (70 mL) thus yielding pure γ-butyrolactone *rac*-**4g**. In turn, the remaining filtrate was quenched with cold 1 % aqueous solution of $NaHCO_3$ (100 mL), extracted with Et_2O (3 × 50 mL), and the combined organic layer was additionally washed with H_2O (100 mL), dried over anhydrous $MgSO_4$ and chromatographed on SiO_2 in accordance to the above-mentioned procedure affording γ-hydroxy ester *rac*-**3g** and residual γ-butyrolactone *rac*-**4g**].

Methyl 4-hydroxy-4-phenylbutanoate (*rac*-3a). Yield 32% (321 mg); colorless oil; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.31; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.99–2.16 (m, 2H), 2.34–2.52 (m, 2H), 3.66 (s, 3H), 4.65–4.81 (m, 1H), 7.24–7.30 (m, 1H), 7.31–7.37 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 30.4, 33.8, 51.7, 73.5, 125.7, 127.6, 128.5, 144.0, 174.3; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{15}O_3^+$ m/z: 195.1016, Found 195.1558, [M+Na] Calcd for $C_{11}H_{14}NaO_3$ m/z: 217.0841, Found 217.1475, $[M+H-H_2O]^+$ Calcd for $C_{11}H_{13}O_2^+$ m/z: 177.0911, Found 177.0959; FTMS (ESI-TOF) *m/z*: $[M+H-H_2O]^+$ Calcd for $C_{11}H_{13}O_2^+$ m/z: 177.09101, Found 177.09114; GC [200–260 (10 °C/min)]: t_R=2.06 min; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=220 nm (Chiralcel OD-H)]: t_R=13.193 and 13.775 min.

5-Phenyldihydrofuran-2(3*H*)-one (*rac*-4a). Yield 23% (192 mg); colorless oil; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.71; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.08–2.25 (m, 1H), 2.55–2.74 (m, 3H), 5.49 (dd, J=7.8, 6.4 Hz, 1H), 7.28–7.46 (m, 5H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 29.0, 30.9, 81.2, 125.3, 128.4, 128.8, 139.4, 176.9; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_{11}O_2^+$ m/z: 163.0754, Found 163.1138, [M+Na] Calcd for $C_{10}H_{10}NaO_2$ m/z: 185.0578, Found 185.0833; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_{11}O_2^+$ m/z: 163.07536, Found 163. 07545; GC [200–260 (10 °C/min)]: t_R=2.26 min; HPLC [*n*-hexane-*i*-PrOH (85:15, v/v); f=0.8 mL/min; λ=210 nm (Chiralcel OD-H)]: t_R=16.033 (*R*-isomer) and 17.239 (*S*-isomer) min or [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=210 nm (Chiralcel OD-H)]: t_R=20.658 (*R*-isomer) and 22.349 (*S*-isomer) min or [*n*-hexane-*i*-PrOH (80:20, v/v); f=1.5 mL/min; λ=210 nm (*S*,*S*-Whelk-O 1)]: t_R=5.823 (*R*-isomer) and 6.637 (*S*-isomer) min.

Methyl 4-(4-fluorophenyl)-4-hydroxybutanoate (*rac*-3b). Yield 56% (617 mg); colorless oil; R_f [CH_2Cl_2/AcOEt (90:10 v/v v/v)] 0.31; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.98–2.07 (m, 2H), 2.38–2.45 (m, 2H), 3.66 (s, 3H), 4.73 (t, J=6.4 Hz, 1H), 6.99–7.05 (m, 2H), 7.28–7.33 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 30.4, 34.0, 51.9, 73.0, 115.4 (d, J_{C-F}=21.5 Hz), 127.5 (d, J_{C-F}=7.9 Hz), 139.9 (d, J_{C-F}=2.9 Hz), 162.3 (d, J_{C-F}=245.6 Hz), 174.4; ^{19}F NMR (470 MHz, $CDCl_3$): δ –115.48 (m); MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{14}FO_3^+$ m/z: 213.0922, Not Found, $[M+H-H_2O]^+$ Calcd for $C_{11}H_{12}FO_2^+$ m/z: 195.0816, Found 195.0816; FTMS (ESI-TOF) *m/z*: $[M+H-H_2O]^+$ Calcd for $C_{11}H_{12}FO_2^+$ m/z: 195.08158, Found 195.08168; GC [200–260 (10 °C/min)]: t_R=1.97 min; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=220 nm (Chiralcel OD-H)]: t_R=10.445 and 11.624 min or [*n*-hexane-*i*-PrOH (95:5, v/v); f=0.8 mL/min; λ=220 nm (Chiralcel OD-H)]: t_R=17.542 and 20.095 min.

5-(4-Fluorophenyl)dihydrofuran-2(3*H*)-one (*rac*-4b). Yield 21% (199 mg); colorless oil; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.75; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.10–2.23 (m, 1H), 2.61–2.70 (m, 3H), 5.48 (dd, J=8.3, 5.9 Hz, 1H), 7.04–7.11 (m, 2H), 7.28–7.35 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 29.1, 31.1, 80.8, 115.8 (d, J_{C-F}=21.5 Hz), 127.3 (d, J_{C-F}=8.8 Hz), 135.2 (d, J=2.9 Hz), 161.8 (d, J=247.9 Hz), 176.7; ^{19}F NMR (470 MHz, $CDCl_3$): δ –

113.80 (m); MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_{10}FO_2^+$ m/z: 181.0660, Found 181.0973; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_{10}FO_2^+$ m/z: 181.06593, Found 181.06602; GC [200–260 (10 °C/min)]: t_R=2.17 min; HPLC [*n*-hexane-*i*-PrOH (85:15, v/v); f=0.8 mL/min; λ=210 nm]: t_R=14.876 (*R*-isomer) and 16.038 min (*S*-isomer) or [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=210 nm (Chiralcel OD-H)]: t_R=19.536 (*R*-isomer) and 21.294 (*S*-isomer) min or [*n*-hexane-*i*-PrOH (80:20, v/v); f=1.5 mL/min; λ=210 nm (*S*,*S*-Whelk-O 1)]: t_R=6.065 (*R*-isomer) and 6.730 (*S*-isomer) min.

Methyl 4-(4-chlorophenyl)-4-hydroxybutanoate (*rac*-3c). Yield 52% (620 mg); colorless oil; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.36; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.98–2.05 (m, 2H), 2.42 (td, *J*=7.2, 2.5 Hz, 2H), 2.56 (br. s., 1H), 3.66 (s, 3H), 4.73 (t, *J*=6.4 Hz, 1H), 7.25–7.33 (m, 4 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 30.4, 33.9, 51.9, 72.9, 127.3, 128.7, 133.4, 142.7, 174.5; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{14}ClO_3^+$ m/z: 229.0626, Not Found, $[M+H–H_2O]^+$ Calcd for $C_{11}H_{12}ClO_2^+$ m/z: 211.0521, Found 211.0645; FTMS (ESI-TOF) *m/z*: $[M+H–H_2O]^+$ Calcd for $C_{11}H_{12}ClO_2^+$ m/z: 211.05203, Found 211.05206; GC [200–260 (10 °C/min)]: t_R=3.09 min; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=220 nm (Chiralcel OD-H)]: t_R=10.863 and 12.669 min.

5-(4-Chlorophenyl)dihydrofuran-2(3*H*)-one (*rac*-4c). Yield 10% (101 mg); white solid; mp 54–56 °C (CH_2Cl_2/AcOEt) [lit.[50] 51 °C (petroleum ether)]; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.76; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.07–2.22 (m, 1H), 2.58–2.73 (m, 3H), 5.43–5.51 (m, 1H), 7.24–7.30 (m, 2H), 7.33–7.40 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 29.0, 31.0, 80.5, 126.8, 129.1, 134.4, 138.0, 176.63; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_{10}ClO_2^+$ m/z: 197.0364, Found 197.0844; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_{10}ClO_2^+$ m/z: 197.03638, Found 197.03657; GC [200–260 (10 °C/min)]: t_R=3.40 min; HPLC [*n*-hexane-*i*-PrOH (98:2, v/v); f=1.2 mL/min; λ=210 nm (Chiralcel OD-H)]: t_R=33.745 (*R*-isomer) and 35.919 (*S*-isomer) min or [*n*-hexane-*i*-PrOH (80:20, v/v); f=1.5 mL/min; λ=210 nm (*S*,*S*-Whelk-O 1)]: t_R=6.038 (*R*-isomer) and 6.799 (*S*-isomer) min.

Methyl 4-(4-bromophenyl)-4-hydroxybutanoate (*rac*-3d). Yield 60% (851 mg); colorless oil; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.36; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.00–2.09 (m, 2H), 2.37 (br. s., 1H), 2.44 (td, *J*=7.1, 3.2 Hz, 2H), 3.68 (s, 3H), 4.69–4.80 (m, 1H), 7.21–7.25 (m, 2H), 7.45–7.50 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 30.4, 33.9, 52.0, 73.0, 121.5, 127.6, 131.8, 143.2, 174.4; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{14}BrO_3^+$ m/z: 273.0121, Not Found, $[M+H–H_2O]^+$ Calcd for $C_{11}H_{12}BrO_2^+$ m/z: 255.0016, Found 255.0375; FTMS (ESI-TOF) *m/z*: $[M+H–H_2O]^+$ Calcd for $C_{11}H_{12}BrO_2^+$ m/z: 255.00152, Found 255.00162; GC [200–260 (10 °C/min)]: t_R=3.86 min; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=220 nm (Chiralcel OD-H)]: t_R=11.642 and 13.443 min.

5-(4-Bromophenyl)dihydrofuran-2(3*H*)-one (*rac*-4d). Yield 11% (134 mg); white solid; mp 89–90 °C (CH_2Cl_2/AcOEt) [lit.[51] 81.5–82.5°C ($CHCl_3$/petroleum ether)]; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.75; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.08–2.21 (m, 1H), 2.61–2.72 (m, 3H), 5.43–5.49 (m, 1H), 7.18–7.24 (m, 2H), 7.49–7.56 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 29.0, 31.0, 80.5, 122.5, 127.1, 132.1, 138.6, 176.6; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_{10}BrO_2^+$ m/z: 240.9859, Found 241.0618, [M+Na] Calcd for $C_{10}H_9BrNaO_2$ m/z: 262.9684, Found 263.0383; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_{10}BrO_2^+$ m/z: 240.98587, Found 241.98605; GC [200–260 (10 °C/min)]: t_R=4.25 min; HPLC [*n*-hexane-*i*-PrOH (80:20, v/v); f=1.5 mL/min; λ=210 nm (*S*,*S*-Whelk-O 1)]: t_R=6.146 (*R*-isomer) and 6.945 (*S*-isomer) min.

Methyl 4-(4-methylphenyl)-4-hydroxybutanoate (*rac*-3e). Yield 34% (366 mg); colorless oil; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.45; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.00–2.10 (m, 2H), 2.14 (br. s., 1H), 2.34 (s, 3H), 2.42 (t, *J*=7.3 Hz, 2H), 3.66 (s, 3H), 4.64–4.76 (m, 1H), 7.12–7.19 (m, 2H), 7.20–7.26 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.2, 30.6, 33.9, 51.8, 73.5, 125.8, 129.3, 137.5, 141.2 174.5; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{17}O_3^+$ m/z: 209.1173, Not Found, $[M+H–H_2O]^+$ Calcd for $C_{12}H_{15}O_2^+$ m/z: 191.1067, Found 191.1080; FTMS (ESI-TOF) *m/z*: $[M+H–H_2O]^+$ Calcd for $C_{12}H_{15}O_2^+$ m/z: 191.10666, Found 191.10677; GC [200–260 (10 °C/min)]: t_R=2.45 min; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=220 nm (Chiralcel OD-H)]: t_R=12.132 and 12.910 min.

5-(4-Methylphenyl)dihydrofuran-2(3*H*)-one (*rac*-4e). Yield 18% (164 mg); white solid; mp 70–71 °C (CH_2Cl_2/AcOEt) [lit.[52] 70–71°C (CH_2Cl_2)]; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.76; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.13–2.25 (m, 1H), 2.36 (s, 3H), 2.57–2.70 (m, 3H), 5.45–5.50 (m, 1H), 7.17–7.25 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.2, 29.1, 31.0, 81.4, 125.5, 129.5, 136.4, 138.4, 177.1; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{13}O_2^+$ m/z: 177.09101, Found 177.1155; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{13}O_2^+$ m/z: 177.09101, Found 177. 09114; GC [200–260 (10 °C/min)]: t_R=2.63 min; HPLC [*n*-hexane-*i*-PrOH (90:10, v/v); f=0.8 mL/min; λ=210 nm (Chiralcel OD-H)]: t_R=16.246 (*R*-isomer) and 17.978 (*S*-isomer) min or [*n*-hexane-*i*-PrOH (80:20, v/v); f=1.5 mL/min; λ=210 nm (*S*,*S*-Whelk-O 1)]: t_R=5.648 (*R*-isomer) and 6.773 (*S*-isomer) min.

Methyl 4-(4-methoxyphenyl)-4-hydroxybutanoate (*rac*-3f). Yield 22% (262 mg); white solid; mp 40–42 °C (CH_2Cl_2/AcOEt) [lit.[53] 41–43°C (Et_2O/petroleum ether)]; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.22; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.99–2.11 (m, 3H, C*H*$_2$ and O*H*), 2.39–2.44 (m, 2H), 3.66 (s, 3H), 3.80 (s, 3H), 4.69 (dd, *J*=7.8, 5.4 Hz, 1H), 6.86–6.90 (m, 2H), 7.24–7.30 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 30.6, 33.9, 51.8, 55.4, 73.3, 114.0, 127.2, 136.3, 159.3, 174.4; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{17}O_4^+$ m/z: 225.1122, Not Found, $[M+H-H_2O]^+$ Calcd for $C_{12}H_{15}O_3^+$ m/z: 207.1016, Found 207.1030; FTMS (ESI-TOF) *m/z*: $[M+H–H_2O]^+$ Calcd for $C_{12}H_{15}O_3^+$ m/z: 207.10157, Found 207.10168; GC [200–260 (10 °C/min)]: t_R=3.53 min; HPLC [*n*-hexane-*i*-PrOH (95:5, v/v); f=0.8 mL/min; λ=220 nm (Chiralpak AD-H)]: t_R=31.816 and 32.984 min.

5-(4-Methoxyphenyl)dihydrofuran-2(3*H*)-one (*rac*-4f). Yield 20% (196 mg); white solid; mp 58–59 °C (CH_2Cl_2/AcOEt) [lit.[53] 55–57°C (Et_2O/petroleum ether)]; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.71; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.14–2.25 (m, 1H), 2.56–2.68 (m, 3H), 3.81 (s, 3H), 5.45 (dd, *J*=8.3, 6.1 Hz, 1H), 6.89–6.93 (m, 2H), 7.23–7.28 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 29.3, 31.0, 55.4, 81.5, 114.2, 127.1, 131.3, 159.9, 177.0; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{13}O_3$+ m/z: 193.0860, Found 193.0984, [M+Na] $C_{11}H_{12}NaO_3$ m/z: 215.0684, Found 215.1066; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{13}O_3$+ m/z: 193.08592, Found 193.08610; GC [200–260 (10 °C/min)]: t_R=3.89 min; HPLC [*n*-hexane-EtOH (99:1, v/v); f=1.0 mL/min; λ=210 nm (Chiralcel OD-H)]: t_R=52.851 (*R*-isomer) and 55.599 (*S*-isomer) min or [*n*-hexane-*i*-PrOH (80:20, v/v); f=1.5 mL/min; λ=210 nm (*S*,*S*-Whelk-O 1)]: t_R=9.183 (*S*-isomer) and 11.228 (*R*-isomer) min.

Methyl 4-hydroxy-4-(pyren-1-yl)butanoate (*rac*-3g). Yield 10% (163 mg); brownish solid; mp 65–66 °C (CH_2Cl_2/AcOEt); R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.36; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.22–2.39 (m, 3H, C*H*$_2$ and O*H*), 2.48–2.69 (m, 2H), 3.69 (s, 3H), 5.84 (dd, *J*=8.2, 4.0 Hz, 1H), 7.97–8.11 (m, 4H), 8.13–8.22 (m, 4H), 8.27–8.35 (d, *J*=9.3 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 30.6, 33.8, 51.9, 70.3, 122.4, 123.2, 124.9, 125.1, 125.2, 125.4, 126.1, 127.4, 127.5, 127.6, 127.9, 130.8, 130.8, 131.5, 137.7, 174.6; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{21}H_{19}O_3^+$ m/z: 319.1329, Not Found, $[M+H–H_2O]^+$ Calcd for $C_{21}H_{17}O_2^+$ m/z: 301.1224, Found 301.1976; FTMS (ESI-TOF) *m/z*: $[M+H–H_2O]^+$ Calcd for $C_{21}H_{17}O_2^+$ m/z:

301.12231, Found 301.12220; GC [260 (const.)]: t_R=Not Found; HPLC [*n*-hexane-*i*-PrOH (78:22, v/v); f=1.0 mL/min; λ=220 nm (Chiralpak AD-H)]: t_R=8.371 and 9.088 min.

5-(Pyren-1-yl)oxolan-2-one (*rac*-4g). Yield 46% (685 mg); yellow solid; mp 170–171 °C (CH_2Cl_2/AcOEt) [lit.[54] 178 °C (AcOH)]; R_f [CH_2Cl_2/AcOEt (90:10 v/v)] 0.78; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.27–2.42 (m, 1H), 2.67–2.83 (m, 2H), 2.90–3.02 (m, 1H), 6.52 (t, J=7.2 Hz, 1H), 8.01–8.12 (m, 6H) 8.15–8.23 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 28.9, 31.0, 79.1, 121.7, 121.8, 124.8, 124.9, 125.1, 125.5, 125.8, 126.3, 127.1, 127.5, 127.8, 128.4, 130.6, 131.3, 131.4, 132.6, 177.3; MS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{20}H_{15}O_2^+$ m/z: 287.1067, Found 287.1706; FTMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{20}H_{15}O_2^+$ m/z: 287.10666, Found 287.10654; GC [260 (const.)]: t_R=Not Found; HPLC [*n*-hexane-*i*-PrOH (78:22, v/v); f=1.0 mL/min; λ=254 nm (Chiralpak AD-H)]: t_R=9.673 and 10.268 min.

General procedure for the analytical-scale studies on stereoselective bioreduction of methyl 4-oxo-4-phenylbutanoate (2a) with different whole-cell biocatalysts – screening procedure

Method A: Each of the lyophilized whole-cell biocatalysts including wild type microorganisms and/or *E. coli* with overexpressed recombinant ADHs (10 mg) were suspended in the reaction solution (500 µL), containing 20 mM glucose, catalytic amounts of the respective cofactors [0.5 mM NADH, 0.5 mM NADPH], and 10 mM substrate **2a** in a mixture of 0.1 M Tris-HCl buffer (pH 7.5)/2-PrOH (500 µL; 90:10, v/v). Biotransformations were conducted in a glass vials (V = 1.5 mL) without air access for 20 h at 30 °C using a laboratory shaker (250 rpm). After this time, the reaction was stopped by extracting the content of the vial with AcOEt (3 × 1 mL), the combined organic phase was dried over anhydrous $MgSO_4$, the filtrate was additionally centrifuged (5 min, 6000 rpm), and only then the supernatant was transferred into a HPLC vial. After the determination of the conversion using GC analyses the solvent was evaporated under reduced pressure, the oil residue was re-dissolved in HPLC-grade 2-PrOH (1.5 mL), and finally, to establish enantiomeric excesses of optically active product **4a** the samples were analyzed by HPLC on a chiral stationary phase (see Supporting Information, **Table S5**). For additional data see also **Table 1**.

Method B: To a suspension of wet *E. coli*/(*S*)-PED cell mass (220 mg wet mass, 49 mg after drying, 2.9 mU/mg assayed with **2a**) in the mixture of 0.1 M MES-KOH buffer (pH 5.5)/2-PrOH (850 µL, 40:60, v/v) a concentrated solution of NADH in MES buffer/2-PrOH mixture (100 µL, 40:60, v/v) was added. The reaction was initiated by adding a concentrated substrate **2a** solution in 2-PrOH (50 µL). The final concentration of the substrate **2a** and NADH in the reaction mixture were 10 mM and 0.5 mM, respectively. The rest of the manipulations are presented below in section named: 'Procedures for Method B and C'.

Method C: The solutions of pure enzymes from *Aromatoleum aromaticum*, that are: (*S*)-HPED (concentration: 3.7 mg/mL, activity 14.5 ±0.6 U/mL assayed with acetophenone, 1.25 ±0.03 U/mL assayed with **2a**) and (R)-HPED (concentration: 4.4 mg/mL, activity 81.7 ±5.2 U/mL assayed with acetophenone; 2.82 ±0.03 U/mL assayed with **2a**) were used for testing reactions. The respective enzyme solution (60 µL) was added to 0.1 M MES-KOH buffer (pH 5.5, 740 µL). Next, a concentrated solution of NADH in the same MES aqueous buffer (100 µL each) and portion of 2-PrOH (50 µL) were added at once. The reaction was initiated by adding the concentrated substrate **2a** solution in 2-PrOH (50 µL). The final concentration of **2a** and NADH in the reaction mixture were 10 mM and 0.5 mM, respectively. The rest of the manipulations are presented below in section named: 'Procedures for Method B and C'.

Procedures for Method B and C: The thus composed biocatalytic reaction systems was shaken in sample tube for 20 h at 30 ºC and 250 rpm. After this time, the reaction was stopped by extraction with AcOEt (3 × 2 mL). The organic layer was separated, dried ($MgSO_4$), the drying agent was filtered off, the filtrate was additionally centrifuged (5 min, 6000 rpm), and only then the supernatant was transferred into a vial to perform GC analysis in order to establish the % conv. After evaporation of the solvent (AcOEt) under reduced pressure, the crude reaction mixture was re-dissolved in CH_2Cl_2 (0.5 mL), followed by the addition of 1-2 drops of trifluoroacetic acid (TFA). The stirring for 1 h at 30 °C completed the lactonization process, and the reaction was further worked-up with saturated aqueous solution of $NaHCO_3$ (3 × 1.0 mL). Next, the organic layer was diluted with CH_2Cl_2 (1.0 mL), washed with brine (1.0 mL) and dried over anhydrous $MgSO_4$. Afterwards, the drying agent was filtered off, the filtrate was centrifuged (5 min, 6000 rpm), and the resulting supernatant was concentrated under vacuum to yield the respective optically active γ-butyrolactone **4a**. After evaporation of the volatiles at reduced pressure by means of rotary evaporator and high-vacuum oil pump (at p=0.05 mmHg), the crude mixture was re-dissolved in 2-PrOH (1.0 mL) and subjected directly to chiral HPLC to establish % ee-values. For additional data see **Table 1**.

General procedure for the analytical-scale studies on stereoselective bioreduction of methyl 4-oxo-4-phenylbutanoate (2a) with different whole-cell biocatalysts – optimization procedure

Lyophilized cells (10 mg) containing overexpressed ADHs were rehydrated in 0.1 M Tris-HCl buffer (pH 7.5, 300 µL) and the solution of 0.5 mM NAD(P)H in the same aqueous buffer (50 µL each) for 30 min at 30 ºC and 250 rpm on a rotary shaker in an glass vial (V = 1.5 mL). Then, 20 mM glucose in Tris-HCl buffer solution (50 µL) and 10 mM substrate **2a** solution in 2-PrOH (50 µL) were added. [*Attention*: in the case of the reactions carried out without addition of NADPH or without both NADPH and glucose or without both cofactors and glucose the missing volumes of the reaction media were supplemented with 50 µL of 100 µL or 450 µL of 0.1 M Tris-HCl buffer (pH 7.5), respectively]. The thus composed biocatalytic reaction system was shaken at 30 ºC and 250 rpm for 48 h (in the case of: *Arthrobacter* sp. DSM 7325) and for 20 h [in the case of: *E. coli*/Lk-ADH-Lica, *E. coli*/RasADH, and *E. coli*/SyADH]. After this time, the reaction was stopped by extraction with AcOEt (3 × 1 mL), the organic layer was combined and washed with brine (1 mL), dried ($MgSO_4$), the drying agent was filtered off, and the filtrate was additionally centrifuged (5 min, 6000 rpm). Finally, the supernatant was transferred into a HPLC vial and the rest of the manipulations for the determination of reaction conversion and % ee-values of optically active product **4a** were the same as described above. For additional data see **Table 2**.

General procedure for the analytical-scale studies on stereoselective bioreduction of methyl 4-oxo-4-arylbutanoates (2a–g) with *E. coli*/RasADH and *E. coli*/SyADH

To a suspension of the respective freeze-dried *E. coli* cells containing the recombinant ADH (*E. coli*/RasADH, and *E. coli*/SyADH, 10 mg each) in 0.1 M Tris-HCl buffer (pH 7.5, 450 µL), the appropriate stock solution of substrate **2a–g** in 2-PrOH (50 µL) to reach 10 mM final concentration was added and the reaction was shaken at 30 ºC and 250 rpm for 20 h (see **Method A** above). The following experimental procedures were the same as before. For additional data see **Table 3**.

General procedure for the analytical-scale studies on stereoselective bioreduction of methyl 4-oxo-4-arylbutanoates (2a–g) with *E. coli*/(*S*)-PED or (*R*)-HPED

The following experimental procedures were the same as for the reactions catalyzed by *E. coli*/(*S*)-PED (see **Method B** above) or (*R*)-HPED (see **Method C** above). The only difference is that the (*R*)-HPED-mediated bioreductions of **2a**–**g** were performed for longer time period (40 h) than in the previous experiments, and in two variants – with 0.5 mM NADH final concentration (**Method C**) or with 1.0 mM NADH final concentration (**Method D**). The analyses of the samples were performed on GC and HPLC after routine work-up of the crude reaction mixtures using AcOEt and subsequent derivatization toward the respective optically active lactone by means of TFA/CH_2Cl_2-based procedure. For additional data see **Table 3**.

Enzyme activity

The enzyme activity unit (U) was defined as the amount of the enzyme which converts 1 μmol of substrate (acetophenone or γ-keto ester **2a**) into the respective product per minute at 30 °C. For details see Supporting Information (section 1.10).

Enzyme concentration measurement

Protein concentrations were determined by the Coomassie dye binding assay according to Bradford protein assay[55] using Sigma Aldrich Bradford protocol.[56] For details see Supporting Information (section 1.11).

HPLC measurements

The reagent concentrations were determined with HPLC using Agilent 1100 system equipped with a DAD detector. The separations were performed on Ascentic® RP-Amide Express column (75 mm × 4.6 mm, 2.7 μm) at 40 ˚C with the isocratic mobile phase composed of H_2O/CH_3CN (65:35, v/v), the flow rate of 1 ml/min and injection volumes of 5 μL. The quantitation of substrate was conducted at 205 nm against external standard calibration. Retention time (t_R) of **2a** was 2.93 min.

Representative procedure for 0.6 mmol scale *E. coli*/(*S*)-PED-catalyzed bioreduction of methyl 4-oxo-4-phenylbutanoate (2a)

A solution of methyl 4-oxo-4-phenylbutanoate (**2a**, 115.3 mg, 0.6 mmol, final concentration 10 mM) in 2-PrOH (3 mL) was added to a suspension of wet cells of *E. coli*/(*S*)-PED (13.2 g, 2.9 mU/mg of wet cell mass of *E. coli*/(*S*)-PED assayed with **2a**, 910.6 mU/mg assayed with acetophenone) and NADH (21.3 mg, 30.0 μmol, final concentration 0.5 mM) in the mixture of 0.1 M MES-KOH buffer (pH 5.5)/2-PrOH (57 mL, 40:60, v/v). [Attention: substrate **2a** and NADH were added from the appropriately prepared stock solutions in 2-PrOH or aqueous buffer, respectively]. The thus composed biocatalytic reaction system was shaken at 30 ºC and 250 rpm for 40 h. After this time, the reaction was stopped by extraction with AcOEt (3 × 120 mL), the combined organic layers dried over anhydrous $MgSO_4$, and after filtering the drying agent under reduced pressure and evaporation of the solvent using rotavap the crude oil was re-dissolved in CH_2Cl_2 (30 mL), followed by the addition of 5 drops of trifluoroacetic acid (TFA). The stirring for 1 h at 30 °C completed the lactonization process, and the reaction mixture was further worked-up with saturated aqueous solution of $NaHCO_3$ (3 × 60 mL). Next, the organic layer was diluted with CH_2Cl_2 (60 mL), washed with brine (60 mL) and dried ($MgSO_4$). Afterwards, the drying agent was removed by filtration, the solvent was evaporated, and the crude residue was subjected on a silica gel column chromatography eluting with a mixture of CH_2Cl_2/AcOEt (90:10, v/v) to afford optically active γ-phenyl-γ-butyrolactone (*S*)-(–)-**4a** [72 mg, 0.44 mmol, 74% yield, >99% ee, $[\alpha]_D^{26}$ = –35.00 (*c* 1.00, $CHCl_3$), lit.[28b] $[\alpha]_D^{23}$ = –34.10 (*c* 1.00, $CHCl_3$, 99% ee)] as yellowish oil. For additional data see **Table 4**.

Procedure for 4.2 mmol scale fed-batch *E. coli*/(*S*)-PED)-catalyzed bioreduction of methyl 4-oxo-4-phenylbutanoate (2a)

A solution of methyl 4-oxo-4-phenylbutanoate (**2a**, 404.7 mg, 2.1 mmol) in 2-PrOH (3 mL) was added to a suspension of wet cells of *E. coli*/(*S*)-PED (46.2 g, 2.9 mU/mg assayed with **2a**, 910.6 mU/mg assayed with acetophenone) and NADH (74.5 mg, 105.0 μmol, final concentration 0.5 mM) in the mixture of 0.1 M MES-KOH buffer (pH 5.5)/2-PrOH (210 mL, 40:60, v/v). The reaction mixture was shaken at 30 ºC and 250 rpm. The concentration of the substrate **2a** was directly monitored using HPLC in 20 h intervals. After 20 h of the reaction we observed almost complete depletion of the substrate **2a** (0.15 mM remained). Therefore, a second portion of methyl 4-oxo-4-phenylbutanoate (**2a**, 404.7 mg, 2.1 mmol) in 2-PrOH (3 mL) was added. The reaction was continued for additional 60 h (i.e. 80 h of total reaction time), after which the substrate **2a** concentration reached 1.58 mM according to HPLC indications. Finally, the reaction was terminated by extraction with AcOEt (3 × 400 mL). The combined organic layers were dried over anhydrous $MgSO_4$, and after filtering off the drying agent under suction and evaporation of the volatiles, the residual oil was re-dissolved in CH_2Cl_2 (50 mL), followed by the addition of 10 drops of trifluoroacetic acid (TFA). The stirring for 5 h at 30 °C completed the lactonization process, and the reaction mixture was further worked-up with saturated aqueous solution of $NaHCO_3$ (3 × 70 mL). Next, the organic layer was diluted with CH_2Cl_2 (80 mL), washed with brine (70 mL) and dried ($MgSO_4$). After evaporation of the solvent on rotavapor the crude product (683 mg) was chromatographed on SiO_2 (50 g) using gradient of CH_2Cl_2/AcOEt (100, 98:2, 95:5, v/v) mixture as an eluent to afford optically active γ-phenyl-γ-butyrolactone (*S*)-(–)-**4a** (457 mg, 2.82 mmol, 67% yield, >99% ee) as yellowish oil. For additional data see **Table 4**.

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FULL PAPER

Biocatalytic Asymmetric Reduction of γ-Keto Esters to Access Optically Active γ-Aryl-γ-butyrolactones

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